precisely control intracellular Ca^{2+} concentrations by targeted photolytic calcium uncaging. We continuously follow Ca^{2+} changes using a radiometric florescent dye fura-2FF. The combination of these techniques provides unprecedented time-resolution to C. elegans neurobiology, and enables us to uncover molecular regulations on rapid exocytosis and endocytosis in individual neuron.

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Interaction of Munc18C with Syntaxin4 and the Role of Munc18C in Exocytosis

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The Sec1/Munc18 (SM) protein family plays an essential role in vesicle fusion processes of eukaryotic cells. The SM proteins are suggested to function in the regulation of SNARE-mediated vesicle fusion, primarily by binding to the SNARE protein syntaxin (Syx). Munc18a, the SM protein involved in synaptic exocytosis, binds to the closed conformation as well as the N-peptide of Syx1a, while some other SM proteins are supposed to bind only the N-peptide. Munc18c, a close homolog of Munc18a, is a ubiquitously expressed vertebrate protein that has a preference for Syx4. It is mainly studied in regulated exocytosis of GLUT4 vesicles in response to insulin. The crystal structure of Munc18c is solved in association with the N-peptide of Syx4, but its interaction with rest of the Syx4 protein is not clear. Also, the influence of Munc18c on SNARE complex formation is debated. We now investigate the Munc18c-Syx4 binding mode and its influence on SNARE assembly kinetics using biochemical and biophysical methods. Our analyses indicate that Munc18c also interacts with the ''closed conformation'' of Syx4. Moreover, the presence of Munc18c slows down the SNARE assembly reaction. Vertebrates have three Munc18 isoforms involved in exocytosis in different tissues that interact with four secretory syntaxin isoforms, but their interaction patterns are not mapped clearly. Since SM proteins are suggested to give specificity to the SNARE interaction, the preference of the secretory SM proteins for the syntaxin isoforms was also studied.

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In Vitro Palmitoylation and Oxidation of the Snare Protein SNAP-25

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The SNARE protein SNAP-25 plays a critical role in neuronal exocytosis. The linker region of SNAP-25 contains 4 cysteines (cysteine-rich domain) which are the sites of various post-translational modifications, such as palmitoylation and oxidation. Palmitoylation anchors SNAP-25 to the membrane (and determines its distribution within the cell), while the level of oxidation may regulate neurotransmitter release.

Quantifying the extent of oxidation and palmitoylation *in vivo* has proven elusive. Our *in vitro* data from SNAP-25 suggests that non-enzymatic palmitoylation is cooperative with a Hill Coefficient greater than 2, whereas oxidation appears to have a Hill coefficient of 1/2 (consistent with two cysteines being consumed for each oxidation event). Using Circular Dichroism to measure the melting of helical structures of SNAP-25, we show that oxidation destabilizes SNAP-25. Oxidation also destabilizes SNARE complex formation. During *in vitro* palmitoylation, we use Mass Spec analysis to confirm that multiple cysteines are palmitoylated under our conditions. *In vitro* palmitoylation and oxidation have an EC50 of about 50 μM. Through additional experimentation we hope to elucidate the EC50, Hill Coefficient, and extent of reaction for enzyme-catalyzed palmitoylation of the cysteine-rich domain of SNAP-25's linker region and also determine how oxidation and palmitoylation alter SNARE complex formation.

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The C2B Domain of Synaptotagmin-1 and Complexin Reduce the Asynchronous Release Activation

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Nerve-evoked vesicle fusion has been kinetically separated in two components, synchronous and asynchronous. The Ca^{2+}-sensor Synaptotagmin binds the SNARE-complex and drive fusion. Complexin co-evolve with Synaptotagmin, bind the complex and speed the exocytosis. Synaptotagmin controls the Ca^{2+}-dependency of quantal release and synchronicity where Complexin cooperate in this function. Neuronal disruption of Synaptotagmin leaves an asynchronous release with a slight Ca^{2+}-cooperativity suggesting the presence of an asynchronous Ca^{2+}-sensor. The asynchronous sensor is thought to mediate the asynchronous releases observed during burst of activity, low Ca^{2+} or replacing Ca^{2+} by Sr^{2+}. Here we investigated the asynchronous release at the Drosophila glutamatergic synapses. We monitor the differential effects of Ca^{2+}, Sr^{2+} and Cd^{2+} in the evoked release modifying the expression of Synaptotagmin and Complexin at the nerve terminals. In addition, we explored the C2A and C2B Ca^{2+} binding domains mutants of Synaptotagmin. Our data shows that the asynchronous/synchronous release depends inversely with the level of Synaptotagmin and Complexin. Moreover, C2B but not C2A domain of Synaptotagmin reduces the asynchronous/synchronous release. Our work suggests that Complexin may be increasing the accessibility of Synaptotagmin for the SNARE complex by the C2B domain reducing the asynchronous/synchronous release. The Cd^{2+}-sensibility was consistent with the Ca^{2+}-dependency in the release probability discarding major alteration in calcium influx.

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Isoform-Specific Roles of Synaptotagmins in Exocytosis

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Adrenal chromaffin cells release hormones and neuropeptides that are essential to the physiological homeostasis of higher organisms. During this process, secretory granules fuse with the plasma membrane to deliver their cargo to the extracellular space. It was classically thought that fusion was the final regulated step in exocytosis, resulting in total release of granule cargo in a uniform manner. More recent evidence argues for non-uniform outcomes after fusion, where cargo is released with variable kinetics and selectivity. The goal of this study was to identify potential factors that contribute to these different outcomes, with a focus on the role of the calcium-sensing synaptotagmin (Syt) proteins. Two Syt isoforms are expressed in chromaffin cells: Syt-1 and Syt-7. We find that overexpressed and endogenous Syt isoforms rarely co-localize on secretory granules and are differentially responsive to weak and strong depolarizing stimuli. In addition, overexpressed Syt-1 and Syt-7 impose isoform-specific effects on fusion pore expansion and granule cargo release. Syt-7 fusion pores usually remain narrow (or reseal), slowing the dispersal of lumenal cargo proteins and granule membrane proteins. On the other hand, Syt-1 diffuses rapidly away from fusion sites and promotes the release of lumenal cargo proteins. These findings suggest one way in which regulation over chromaffin cell cargo release may be achieved is via selective activation of synaptotagmin isoforms.

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Frictional Additivity of Lateral Diffusion on Supported Bilayers: Influence of Linker Length in Synaptotagmin 7 C2A-C2B Tandem Domains

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The synaptotagmin (Syt) family of proteins contains tandem C2 domains, C2A and C2B, which bind membranes in the presence of Ca^{2+}. The precise mechanism by which Syt participates in membrane fusion events is not completely understood, and the role of interactions between C2A and C2B in membrane binding and fusion is unclear. To test whether the two domains interact with each other on a planar lipid bilayer, diffusion constants of fluorescent-tagged C2A, C2B, and tandem C2AB domains from Syt7 were determined using total internal reflection fluorescence microscopy with single-particle tracking. As expected, the tandem domain diffuses significantly more slowly than the single domains. However, the diffusion constant of the C2AB tandem is significantly faster than predicted by simple summation of the individual domains' frictional coefficients. This result suggests that either (a) the short interdomain linker holds the two domains in close proximity to each other in the plane of the bilayer, at a separation distance shorter than the free draining limit, and/or (b) the two domains directly interact with each other when membrane-bound. In order to distinguish between these two possibilities, several mutant versions of the tandem domain with extended C2A-C2B linker regions were purified. Preliminary results indicate these mutants continue to diffuse similarly to the native C2AB tandem and faster than predicted based on frictional additivity, even at linker distances expected to exceed the free draining limit. These results suggest that C2A and C2B interact with each other when bound to a membrane in a manner that is independent of C2A−C2B linker length. Current experiments in this ongoing project probe the nature